UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG - 5 1988

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of toxicology data for registration of the microbial pesticide control agent Bacillus thuringiensis var. tenebrionis.

TO: W. Nelson (PM-17)
Registration Division

FROM: Roy D. Sjoblad, Ph.D.
Microbiologist, SMSS, Toxicology Branch R. D. Sjoblad 8/3/88

THROUGH: Reto Engler, Ph.D.
Chief, SMSS 8/3/88

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch 8/5/88

Microbial pest control agent: Bacillus thuringiensis var. tenebrionis

Product/trade name: Trident biological insecticide

EPA record No.: 220045 Caswell No.: 66

EPA ID No.: 55947-RGR Tox. Branch Project No.: 8-0689

Action requested: Review Toxicology data provided to support the registration of B. thuringiensis var. tenebrionis.

Conclusion: The Toxicology Branch supports the registration of Trident biological insecticide. The test substance elicited no significant adverse reactions in test animals after oral, intravenous or dermal dosing. Pulmonary exposure was lethally toxic to 2/26 test animals; and test animals had difficulty in clearing the bacterium after pulmonary or intravenous exposure. The test material elicited mild eye and dermal irritation reactions. Additional information on characterization of the test bacterium which should be provided include plasmid size designations, antibiotic resistance profiles, and source and history of the bacterium.

TOX REVIEW 8/5/88

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Summary: The following studies were submitted for review. Toxicology Branch classifications of the studies are indicated in parentheses.

Acute oral toxicity/infectivity-rat	(Acceptable)
Acute pulmonary toxicity/infectivity-rat	(Acceptable)
Acute intravenous toxicity/infectivity- rat	(Acceptable)
Acute dermal toxicity-rabbit	(Acceptable)
Eye irritation/infectivity-rabbit	(Acceptable)

In addition, certain information in the Product Chemistry volume (i.e., taxonomy, and evaluation for beta-exotoxin) is considered pertinent to mammalian toxicology issues, and also is reviewed.

The data support the conclusion that the test material containing B. thuringiensis var. tenebrionis is not pathogenic for nor infective in the test animals when administered in a single high dose by the oral, pulmonary or intravenous routes. The data show that the test material can be lethally toxic to test animals when administered at a single high dose via the intratracheal route of exposure and the possibility of spore germination and subsequent vegetative cell replication cannot be ruled out. The cause of the observed mortality has not been established; and the presence of a microbially-produced toxin cannot be ruled out. The data indicate that the test animals have difficulty in clearing the spores from the lung.

No significant, persistent signs of toxicity were observed in test animals after acute oral or intravenous administration of the test material. Lethargy was observed for up to 24 hours after acute pulmonary exposure to the test substance, and this was attributable to intact spores of B. thuringiensis var. tenebrionis. Both autoclaved and non-autoclaved preparations of the test material caused a mean decrease in body temperature for up to 4 hours after pulmonary exposure.

The test material did not cause any macroscopic lesions in any test animal, upon any route of administration.

The test material was not toxic (Tox. category IV) when applied dermally, and was a mild eye irritant (transient conjunctival irritation only; Tox. category III). The test material also was a skin irritant, with signs of slight erythema and edema persisting for up to 12 days after dosing.

Additional information should be provided to allow for a more complete characterization of the bacterium. These include the source/history of the bacterium, antibiotic resistance patterns, and plasmid size designations.

The Toxicology Branch recommends that appropriate respiratory tract and eye protective coverings be worn when exposure to aerosols of the test material may occur. The product label should reflect these precautions.

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An assessment of the overall results from the data package do not warrant the requirement of additional studies at this time. The data indicate that after intravenous injection, the test animal immune system is effective in slowly processing the spores and eliminating them from the test animals. The data from the intratracheal exposure study indicate that the test animals even have greater difficulty in processing and clearing the bacterial spores from the lungs, after delivery of approximately 10^8 spores/animal. Thus, proper pulmonary tract coverings are warranted during times of potential exposure to aerosols of the test material.

The two intentionally added inert ingredients are cleared according to §180.1001 (See attachment; Confidential business information; statement of formula)

CONFIDENTIAL

INERT INGREDIENT FILE - SHAUGHNESSEY - POTOMAC NUMBERING SYSTEM

NUMERICALLY BY SHAUGHNESSEY NUMBER

Shaughnessey #	Pot #	CAS #	Chemical Identity	Clearance 180.1001

Inert ingredient information may be entitled to confidential treatment

Roy Sjoblad TRIDENT Biological Insecticide

CONFIDENTIAL

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Reviewed by: Roy D. Sjoblad, Ph.D.
SMSS, Toxicology Branch (TS-769C)
Secondary reviewer: Reto Engler, Ph.D.
Chief, SMSS

DATA EVALUATION REPORT

STUDY TYPE: Acute oral study in the rat. TOX. CHEM NO.: 66

ACCESSION NUMBER: MRID NO.: 404974-03

TEST MATERIAL: B. thuringiensis var. tenebrionis

SYNONYMS: Trident; SAN 418-SC-62

STUDY NUMBER: HRC: 871633D/SNC 11/AC; SCPC: 2448/87/P10

SPONSOR: Sandoz Crop Protection Corporation (SCPC)

TESTING FACILITY: Huntingdon Research Centre, Ltd. (HRC); England

TITLE OF REPORT: Acute oral toxicity and infectivity to rats of SAN 418-SC-62

AUTHOR(S): D.J.N. Hossack, J.R. Gardner, M.N. Baker

REPORT ISSUED: November, 1987

CONCLUSIONS: The test article, SAN 418-SC-62, containing 4.8×10^{10} CFU/ ml of B. thuringiensis var. tenebrionis was not toxic to, infective in, or pathogenic for rats when administered in a single oral dose at 20 ml/kg body weight. At study termination (21 days after dosing) generally $<10^2$ bacteria were detected per gram of caecal contents or per gram of feces.

Tox Category: N/A.

Classification: Acceptable.

I. STUDY DESIGN: A. Test material: SAN 418-SC-62, a spore suspension of B. thuringiensis var. tenebrionis (Lot # P15-75), containing 4.8×10^{10} viable spores/ml. An autoclaved (121°C for 30 min) preparation of the spore suspension also was used.

B. Test animals: CD rat [CR1:CD (SD) BR] from Charles River, U.K. Ltd., England; Weight: 96-126 g.

C. Methods: Eleven male and eleven female rats each were dosed orally with the test substance at 20 ml/kg. An infectivity control group of 5 male and 5 female rats each were dosed orally with 20 ml/kg of the autoclaved test material, and an untreated control group of 5 male and 5 female rats also was included. Animals

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were observed at least twice/day for clinical signs of toxicity and illness. Body temperatures of all test animals were determined just prior to dosing, and at 2, and 4, and 24 hours after dosing. Test animal body weights were measured on the day of dosing, and at 7, and 14, and 21 days after dosing. Urine and feces samples were collected from 5 male and 5 female animals dosed with SAN 418-SC-62 at 1, 2, 3, and 21 days after dosing and were analyzed for the test bacterium. Two male and two female rats were sacrificed (cervical dislocation) at 1, and at 7, and at 14 days after dosing with the test substance. The remainder of the dosed animals and all the control group animals were sacrificed at 21 days. All sacrificed animals were subjected to a gross post-mortem examination. The following tissues/body fluids from all sacrificed animals dosed with the viable spore suspension were examined for the presence of the test bacterium: blood, brain, heart, lungs, kidney, liver, spleen, mesenteric lymph nodes, and contents of stomach, small intestine, and caecum. Presumptive determination of B. thuringiensis from organ/tissue samples was made by smearing the surface of one-half of the sample on solid growth medium. A quantitative analysis was done on the remainder of the organ/tissue sample if the presumptive test was positive for the test bacterium.

II. RESULTS

None of the test animals died on-study. The only clinical sign of toxicity was pilo-erection, which was observed for 1 day after dosing in all rats treated with the viable suspension or with the autoclaved suspension of SAN 418-SC-62. Animal body temperatures did not indicate a pyrogenic response to the non-autoclaved or to the autoclaved test materials. There was no significant effect of the test materials on body weight gain by the test animals. The feces of dosed animals contained from approximately 10^9-10^{10} B. thuringiensis CFU/gram at 1 day after dosing, and decreased to approximately 10^6-10^9 at 3 days after dosing. At 21 days after dosing, fecal counts of B. thuringiensis were <75/g for 9/10 animals, and was 175/g for 1/10 animals. Viable bacteria ($<10^1$-10^3) were detected in the urine samples, but, this was most likely due to contamination from the fecal material in the metabolism cages. Viable bacteria were readily detected in the intestinal tract contents at 1 day after dosing, and the data showed that the bacteria became steadily cleared from the stomach, and then from the small intestine, and finally from the caecum as time after dosing lengthened. At 21 days after dosing no B. thuringiensis were detected in the stomach or in the first loop of the intestine, and <100 CFU/g were detected in the caecum of each animal.

At 1 day after dosing, viable B. thuringiensis were detected at 7×10^2 CFU/g of lung tissue from one male rat, and at 3×10^2 CFU/g and at 6.7×10^1/g of lymph node tissue in two other rats. The presumptive test showed that the test bacterium could be detected at low numbers (i.e., 1-50 CFU/tissue smear) in other samples from these animals at the day 1 analysis time.

After the day 1 sample analysis time, only 1 CFU of B. thuringiensis was infrequently detected (i.e., in 5/144 samples)

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in tissue smears from test animals.

III. DISCUSSION:

1. The sporadic appearance of 1 CFU of the test bacterium in tissue sample smears is not sufficient to indicate infectivity, and is not considered unusual, since it most likely is due to contamination by bacteria not directly associated with the tissue samples.

2. It appeared that the pulmonary tracts of several animals were exposed to the bacterium at or around the time of oral dosing. However, analyses of test animals at >24 hours after dosing failed to reveal any significant numbers of pulmonary tract-associated bacteria. An analysis of non-dosed or infectivity control group animals might have been useful for determining the ability of the bacterium to be transmitted from dosed to non-dosed animals.

3. The number of spores administered was approximately 10^{10} per test animal and was sufficient for the purposes of this study.

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Reviewed by: Roy D. Sjoblad, Ph.D. R.D. Sjoblad
SMSS, Toxicology Branch (TS-769C)
Secondary reviewer: Reto Engler, Ph.D.
Chief, SMSS

DATA EVALUATION REPORT

STUDY TYPE: Acute pulmonary study in the rat. TOX. CHEM NO.: 66

ACCESSION NUMBER: MRID NO.: 404974-05

TEST MATERIAL: B. thuringiensis var. tenebrionis

SYNONYMS: Trident; SAN 418-SC-62

STUDY NUMBER: HRC: 871633D/SNC 14/AC; SCPC: 2448/87/P12

SPONSOR: Sandoz Crop Protection Corporation (SCPC)

TESTING FACILITY: Huntingdon Research Centre, Ltd. (HRC); England

TITLE OF REPORT: Acute pulmonary toxicity and infectivity to rats of SAN 418-SC-62

AUTHOR(S): D.J.N. Hossack, J.R. Gardner, M.N. Baker

REPORT ISSUED: December 7, 1987

CONCLUSIONS: A 10% (w/v) preparation of the test article, SAN 418-SC-62, containing 4.8×10^9 CFU/ ml of B. thuringiensis var. tenebrionis was lethally toxic to 2/26 rats when administered in a single intratracheal dose at 1.2 ml/kg body weight. The test bacterium could persist in high numbers in lungs, and was detectable in heart and brain samples of several test animals at 21 days after dosing (i.e., at study termination).

Tox Category: N/A.

Classification: Acceptable.

I. STUDY DESIGN: A. Test material: SAN 418-SC-62, a spore suspension of B. thuringiensis var. tenebrionis (Lot # P15-75), containing 4.8×10^{10} viable spores/ml. An autoclaved (121°C for 30 min) preparation of the spore suspension also was used.

B. Test animals: CD rat [CR1:CD (SD) BR] from Charles River, U.K. Ltd., England; Weight: 210-280 g.

C. Methods: Thirteen male and thirteen female rats each were dosed intratracheally with a 10% w/v preparation of the test substance in physiological saline. The dose level of the diluted test material was 1.2 ml/kg. An infectivity control group of 5 male and 5 female rats each were dosed with 1.2 ml/kg of the autoclaved diluted test material. An untreated control group of 5 male and 5 female rats also was included.

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Animals were observed at least twice/day for clinical signs of toxicity and illness. Body temperatures of all test animals were determined just prior to dosing, and at 2, and 4, and 24 hours after dosing. Test animal body weights were measured on the day of dosing, and at 7, and 14, and 21 days after dosing. Urine and feces samples were collected from 5 male and 5 female animals dosed with 10% w/v SAN 418-SC-62 at 1 and 21 days after dosing and were analyzed for the test bacterium. Two male and two female rats were sacrificed (ether inhalation) at 1 hour, and at 1, 7, and at 14 days after dosing with the test substance. The remainder of the dosed animals and all the control group animals were sacrificed at 21 days. All sacrificed animals and all animals found dead were subjected to a gross post-mortem examination. The following tissues/body fluids from all sacrificed and dead animals dosed with the viable spore suspension were examined for the presence of the test bacterium: blood, brain, heart, lungs, kidney, liver, spleen, and mesenteric lymph nodes. Presumptive determination of B. thuringiensis from organ/tissue samples was made by smearing the surface of one-half of the sample on solid growth medium. A quantitative analysis was done on the remainder of the organ/tissue sample if the presumptive test was positive for the test bacterium.

II. RESULTS

Two male rats died at 2 days after dosing with SAN 418-SC-62. During the day of dosing, the following clinical signs were observed in virtually every test animal treated with the viable suspension or with the autoclaved suspension of SAN 418-SC-62: pilo-erection, hunched posture, waddling, decreased respiratory rate, pallor of extremities, increased salivation, and collapsed state. All test animals dosed with the non-autoclaved test material were lethargic, while none of the animals dosed with the autoclaved test material were lethargic. Non-dosed animals exhibited pilo-erection only. No significant clinical abnormalities were observed after the first day of dosing. Animal body temperatures did not indicate a pyrogenic response to the non-autoclaved or to the autoclaved test materials. A mean body temperature decrease of 0.5-0.8°C was indicated from 2-4 hours after dosing with the non-autoclaved test material, and a decrease of 0.6-2.2°C was indicated at 4 hours after dosing with the autoclaved material. There was no significant effect on body weight gain by that could be attributed to dosing with the test materials. No macroscopic lesions were noted in any test animal upon gross post-mortem examination.

The lungs of dosed animals contained from approximately $5x10^5$-$2x10^6$ B. thuringiensis CFU/gram at 1 hour after dosing, and from $5x10^4$-$2x10^7$ at 24 hours after dosing. At 21 days after dosing, 4/9 rats still had lung counts of B. thuringiensis in excess of 10^5 CFU/g. The remaining four rats at 21 days had from <10-60 CFU/g lung tissue.

Smears of tissues/body fluids indicated that the test bacterium was widely disseminated throughout the test animals, especially at the 1 and 24 hour sampling time. However, even at 7 and 21 days after dosing, the smear-technique indicated the presence of a significant number of B. thuringiensis associated with the heart of 2/9 animals and the brain of 1/9 animals.

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The quantitative isolation of viable B. thuringiensis CFU from tissues and from blood confirmed, in general, the presumptive smear-plate results. At 1 hour after dosing, from 10^2 to 10^3 CFU/g or ml were associated with the lymph nodes and blood from 4/4 animals; from the brain and liver of 3/4 animals; from the heart and kidney of 2/4 animals; and from the spleen of 1/4 animals. At 24 hours after dosing, 10^2-10^3 CFU/g or ml were enumerated from the hearts of 3/4 animals, from the liver and spleen of 2/4 animals, and from the kidney and blood of 1/4 animals. At 7 days after dosing to the final analysis time of 21 days, B. thuringiensis were not detected in lymph nodes, blood, and kidneys of the test animals. The data also showed a trend of clearing of the organism from the other tissues examined. However, approx. $2x10^2$ CFU/g of heart sample were detected in 2/9 animals, and approx. $6x10^2$ CFU/g of brain sample were detected in 1/9 animals.

At 24 hours after dosing, the feces of 10/10 animals examined contained approx. 10^6 CFU/g, and at 21 days after dosing, feces from 10/10 animals contained from 10^2-10^3 CFU/g. In general, animal caecum contents contained $<10^2$ CFU/g at 1 hour, 10^5 CFU/g at 24 hours, 10^4 CFU/g at 7 days, 10^3 CFU/g at 14 days, and from 10^1-10^3 CFU/g at 21 days.

The one rat that died within 24 hours after dosing, and whose tissues and blood were analyzed at the 24 hour time period, showed the highest number of B. thuringiensis CFU in the heart, brain, liver, spleen, and blood, when compared to the other 3 animals analyzed at this time period.

III. DISCUSSION:

1. The dose level of approximately 10^8 B. thuringiensis CFU/test animal was sufficiently high for the purposes of this study.

2. The observed signs of toxicity which included hunched posture, waddling, decreased respiratory rate, pallor of extremities, increased salivation, collapsed state and reduced body temperature, appeared to be due to administration of the test material and not to any property of the viable test bacterium, since both the autoclaved and non-autoclaved test materials elicited these effects. However, an analysis of the autoclaved preparation to verify the effectiveness of the spore-killing procedure was not reported.

3. The observed lethargy in test animals appeared to be an effect elicited solely by the non-autoclaved preparation.

4. The two deaths probably were caused by the test material (SAN 418-SC-62).

5. The bacterium was cleared at a slow rate from the test animals, and the data indicate that clearance of the initial dose of spores from the lungs was a difficult process. The data also did not rule out the possibility of spore germination and subsequent replication.

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Secondary reviewer: Reto Engler, Ph.D.
Chief, SMSS

DATA EVALUATION REPORT

STUDY TYPE: Acute intravenous study in the rat. TOX. CHEM NO.: 66

ACCESSION NUMBER: MRID NO.: 404974-06

TEST MATERIAL: B. thuringiensis var. tenebrionis

SYNONYMS: Trident; SAN 418-SC-62

STUDY NUMBER: HRC: 871680D/SNC 12/AC; SCPC: 2448/87/P11

SPONSOR: Sandoz Crop Protection Corporation (SCPC)

TESTING FACILITY: Huntingdon Research Centre, Ltd. (HRC); England

TITLE OF REPORT: Acute intravenous toxicity and infectivity to rats of SAN 418-SC-62

AUTHOR(S): D.J.N. Hossack, J.R. Gardner, M.N. Baker

REPORT ISSUED: November 18, 1987

CONCLUSIONS: The test article, SAN 418-SC-62, containing 4.8×10^{10} CFU/ ml of B. thuringiensis var. tenebrionis was not toxic to, infective in, or pathogenic for rats when a 1% (w/v) preparation was administered in a single intravenous dose at 3.0 ml/kg body weight. Spores were cleared slowly from the test animals, however, the data are consistent with a normal process of immunological mechanisms leading to clearance. (a)

Tox Category: N/A.

Classification: Acceptable

I. STUDY DESIGN: A. Test material: SAN 418-SC-62, a spore suspension of B. thuringiensis var. tenebrionis (Lot # P15-75), containing 4.8×10^{10} viable spores/ml. An autoclaved (121°C for 30 min) preparation of the spore suspension also was used.

B. Test animals: CD rat [CR1:CD (SD) BR] from Charles River, U.K. Ltd., England; Weight: 92-148 g.

C. Methods: Thirteen male and thirteen female rats each were dosed by intravenous injection into a lateral tail vein with a 1% w/v preparation of the test substance in phosphate-buffered saline.

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The dose volume of the diluted test material was 3.0 ml/kg. An infectivity control group of 5 male and 5 female rats each were dosed with 3.0 ml/kg of the autoclaved diluted test material. An untreated control group of 5 male and 5 female rats also was included.

Animals were observed at least twice/day for clinical signs of toxicity and illness. Body temperatures of all test animals were determined just prior to dosing, and at 2, and 4, and 24 hours after dosing. Test animal body weights were measured on the day of dosing, and at 7, and 14, and 21 days after dosing. Urine and feces samples were collected from 5 male and 5 female animals dosed with 1% w/v SAN 418-SC-62 at 1 and 21 days after dosing and were analyzed for the test bacterium. Two male and two female rats were sacrificed (ether inhalation) at 1 hour, and at 1, 7, and at 14 days after dosing with the test substance. The remainder of the dosed animals and all the control group animals were sacrificed at 21 days. All sacrificed animals were subjected to a gross post-mortem examination. The following tissues/body fluids from all sacrificed animals dosed with the viable spore suspension were examined for the presence of the test bacterium: blood, brain, heart, lungs, kidney, liver, spleen, and mesenteric lymph nodes. Presumptive determination of B. thuringiensis from organ/tissue samples was made by smearing the surface of one-half of the sample on solid growth medium. A quantitative analysis was done on the remainder of the organ/tissue sample if the presumptive test was positive for the test bacterium.

II. RESULTS

No rats died on-study. Within the first five hours after dosing, the following clinical signs were observed in all test animals treated either with the viable suspension or with the autoclaved suspension of SAN 418-SC-62: pilo-erection, pallor of extremities, and increased locomotor activity. Non-dosed animals exhibited pilo-erection only. No significant clinical abnormalities were observed after five hours post-dosing. Animal body temperatures did not indicate a pyrogenic response to the non-autoclaved or to the autoclaved test materials. Mean body temperatures of animals in dosed groups were comparable to the non-treated group. There was no significant effect on body weight gain by that could be attributed to dosing with the test materials. No macroscopic lesions were noted in any test animal upon gross post-mortem examination.

Smear plate results indicated that the test bacterium was associated with tissues and blood of 4/4 test animals at the 1 hour sampling time and with all samples of 3/4 animals at the 24 hour sampling time. At all sample times the smear-plates indicated confluent growth of B. thuringiensis from liver and spleen samples. This technique also indicated that with time, the bacteria were steadily being cleared from the brain, heart, lungs, blood, and lymph nodes.

The quantitative enumeration of viable B. thuringiensis from tissues and blood of test animals generally correlated with the smear-plate data. Blood from test animals contained approximately 10^3 bacteria/ml at 1 and 24 hours after dosing, and virtually no CFU/ml at 14 and 21 days. The brain, heart, lungs, kidneys, and

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lymph nodes also essentially were cleared of bacteria by 14 days after dosing. At 21 days the number of bacteria in spleen was approximately 10-fold less than the 1 hour analysis of approximately 10^6 CFU/g. Also, at 21 days post-dosing, the livers of all test animals contained from approximately $1x10^3$ to $5x10^3$ CFU/g compared to 10^5 CFU/g observed at 1 hour after dosing. Between 20 to 200 CFU/g tissue were observed in the following samples in rats (#/group) at 21 days after dosing: brain (2/10), lungs (3/10), kidney (2/10) and lymph node (3/10).

At 24 hours after dosing, the feces of 10/10 animals examined contained approx. 10^3 CFU/g, and at 21 days after dosing, feces from 10/10 animals contained <25 CFU/g. In general, animal caecum contents contained approx. 10^3 CFU/g at 1 and at 24 hours, and $<10^2$ CFU/g at 21 days.

III. DISCUSSION:

1. The dose level of approximately 10^7 B. thuringiensis CFU/test animal was sufficiently high for the purposes of this study.

2. The data are consistent with a trend for clearance of bacterial spores from the bloodstream, and from the whole animal after intravenous injection. The data also indicate that the test bacterium is not replicating within the test animal or its organs.

3. Spores were continuing to be cleared from the test animals at the 21-day post-dosing study termination time, and association of 10^3 spores/g liver and 10^5 spores/g spleen cannot be considered unusual in the absence of any other signs of illness or toxicity. Detection of viable spores in the caecal contents and feces of test animals at 21 days after dosing indicates that the bacterial spores can be immunologically processed through the animals without necessarily being rendered incapable of germination. This contention, however, is dependent on the assumption that animals are not being re-exposed orally or even by the pulmonary route to the bacterial spores during the study period.

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Reviewed by: Roy D. Sjoblad, Ph.D.
SMSS, Toxicology Branch (TS-769C)
Secondary reviewer: Reto Engler, Ph.D.
Chief, SMSS

DATA EVALUATION REPORT

STUDY TYPE: Acute dermal toxicity study in the rabbit.

ACCESSION NUMBER: TOX CHEM NO.: 66 MRID NO.: 404974-04

TEST MATERIAL: B. thuringiensis var. tenebrionis

SYNONYMS: Trident; SAN 418-SC-62

STUDY NUMBER: HRC: 871596D/SNC 13/E

SPONSOR: Sandoz Crop Protection Corporation (SCPC)

TESTING FACILITY: Huntingdon Research Centre, Ltd. (HRC); England

TITLE OF REPORT: Acute dermal toxicity to rabbits of SAN 418-SC-62

AUTHOR(S): M.P. Liggett, P.A. Mullins, D.J.N. Hossack, M.N. Baker

REPORT ISSUED: October 12, 1987

CONCLUSIONS: The test article, SAN 418-SC-62, containing 4.8×10^{10} CFU/ ml of B. thuringiensis var. tenebrionis was not toxic to rabbits when applied to the skin in a single dose at 2.0 ml/kg body weight for a period of 24 hours. The test substance was a mild skin irritant.

Tox Category: IV

Classification: Acceptable

I. STUDY DESIGN: A. Test material: SAN 418-SC-62, a spore suspension of B. thuringiensis var. tenebrionis (Lot # P15-75), containing 4.8×10^{10} viable spores/ml.

B. Test animals: New Zealand White rabbit, from Froxfield Rabbits, England; Weight: 2.1-2.9 kg.

C. Methods: The test material at 2 ml/kg animal body weight was applied to the clipped area (approx. $10 cm^2$) at the dorso-lumbar region of each test animal. Each treated area was covered with gauze and then with elastic adhesive dressing and finally with waterproof plaster. At 24 hours after treatment, dressings and gauze were removed, and treated areas were washed to remove

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residual test material. Animals were observed for clinical signs of toxicity at least two times daily for 14 days. Treated skin was graded for erythema and eschar formation and for edema at 30 min. after patch removal, and then daily for 14 days. Test animal body weights were measured on the day of dosing, and at 7, and 14, and 21 days after dosing. At 14 days after dosing, all test animals were sacrificed and were were subjected to a gross post-mortem examination.

II. RESULTS

No rats died on-study, and no signs of systemic toxicity were observed in any test animal. There was no significant effect on body weight gain by that could be attributed to dosing with the test materials. No macroscopic treatment-related lesions were noted in any test animal upon gross post-mortem examination.

All test animals showed well-defined erythema and from slight to well-defined edema at 30 minutes after removal of patches and at 24 hours after patch removal. Slight erythema and slight edema persisted in 9/10 test animals at 12 days after patch removal. At 13 days after patch removal, the skin of all test animals appeared normal.

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SMSS, Toxicology Branch (TS-769C)
Secondary reviewer: Reto Engler, Ph.D.
Chief, SMSS

DATA EVALUATION REPORT

STUDY TYPE: Primary eye irritation study in the rabbit.

ACCESSION NUMBER: TOX CHEM NO.: 66 MRID NO.: 404974-07

TEST MATERIAL: B. thuringiensis var. tenebrionis

SYNONYMS: Trident; SAN 418-SC-62

STUDY NUMBER: HRC: 871597D/SNC 15/E

SPONSOR: Sandoz Crop Protection Corporation (SCPC)

TESTING FACILITY: Huntingdon Research Centre, Ltd. (HRC); England

TITLE OF REPORT: Irritant and infective effects on the rabbit eye of SAN 418-SC-62.

AUTHOR(S): M.P. Liggett and D.J.N. Hossack

REPORT ISSUED: October 12, 1987

CONCLUSIONS: The test article, SAN 418-SC-62, containing 4.8×10^{10} CFU/ ml of B. thuringiensis var. tenebrionis was not damaging to eyes of rabbits when applied in a single dose at 0.1 ml/eye. The test substance caused slight conjunctival irritation. No signs of irritation were observed at >3 days after dosing.

Tox Category: III

Classification: Acceptable

I. STUDY DESIGN: A. Test material: SAN 418-SC-62, a spore suspension of B. thuringiensis var. tenebrionis (Lot # P15-75), containing 4.8×10^{10} viable spores/ml.

B. Test animals: New Zealand White rabbit, from Froxfield Rabbits, England; Weight: 2.1-3.0 kg.

C. Methods: The test material at 0.1 ml was placed into the lower everted eyelid of each of 6 test animals. Eyelids were held together for one second before releasing. The contralateral eye of each animal was not treated and served as a control. Eyes were examined prior to dosing to ensure no pre-existing damage. Animals were restrained by Elizabethan collars to minimize

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contamination of untreated eyes. Eyes were examined at 1 hour after treatment, and at 1, 2, 3, 4, 7, 8, 9, 10, 11, and 14 days after treatment. Eyes were scored for ocular lesions according to the system of Draize. To evaluate the presence of the test bacterium, swabs of eyes were taken prior to dosing and at 1, 2, 3, 4, 7, 9, and 14 days after dosing. The eyes of 1/6 rabbits also were swabbed for bacterial enumeration at 16 and 21 days after dosing. In addition, the eyes of 5/6 rabbits were irrigated with sterile physiological saline at 7 days after dosing. The sixth test animal was treated similarly at 14 days after dosing. The irrigating fluid was analyzed for B. thuringiensis.

II. RESULTS

Conjunctival redness, discharge, and chemosis (Draize score: 6-10) were recorded in each treated eye at 1 hour after treatment. These signs of irritation lessened at the 24 hour observation period (Draize score: 4-6). Slight redness was observed in the treated eyes of 2/6 test animals at 2 days after dosing, and in 1/6 animals at 3 days after dosing. No signs of irritation were observed in any test animal eyes from 4 days after dosing until study termination.

Viable Bacillus thuringiensis spores could be isolated from both treated and control eyes, even up to the time of study termination. In general, treated eyes contained higher numbers of viable spores than did the untreated eyes, and washing was effective in reducing the number of B. thuringiensis spores in the eyes. When tested (i.e., at 9 days after dosing), B. thuringiensis also could be enumerated from animals ears and paws.

III. DISCUSSION

Although viable spores could be isolated from eyes of test animals, even at study termination, there was no indication that the resident spores were causing any damage. It appears likely that eyes could be reinoculated from spores associated with other parts of the test animal bodies. There was no evidence that the test bacterium was able to invade and proliferate in animal eyes. It is considered an appropriate safety precaution to wear protective eye coverings when exposure to aerosols of microbial pesticide preparations may occur. The label should include a statement to this effect.

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DATA EVALUATION REPORT

STUDY TYPE: Microorganism taxonomy/characterization TOX. CHEM. NO.: 66

SPONSOR: Sandoz Crop Protection Corporation

TITLE OF REPORT: Trident Biological Insecticide Product Chemistry

AUTHORS: Sandoz CPC; Microbial Research Division

REPORT ISSUED: August 28, 1987

CONCLUSION: Although the information provided allows for the identification of the test bacterium as B. thuringiensis var. tenebrionis. Certain additional data should be provided to allow for better characterization of the test microorganism. These include antibiotic resistance patterns, plasmid size designation, and history, including source of the bacterium.

The purpose of this Data Evaluation Report is to provide review on that information in the Product Chemistry section of the registration package that is pertinent to Toxicology Branch issues. Primarily, the information considered is that which relates to the taxonomy/characterization of the microorganism, presence of mammalian toxins, subcutaneous toxicity to mice, and batch analysis for microbial contaminants.

Information provided:

The bacterium designated as Bacillus thuringiensis var. tenebrionis is a Gram-positive aerobic spore-forming bacterium, which contains a rhomboid-shaped crystalline protein inclusion. Serotype analysis showed the flagellar antigen 8a8b H-serotype. The bacterium was lethal to certain coleopteran insects.

Gel electrophoresis of the crystalline protein showed two major proteins of 74kd and 64kd, and two minor proteins of 39kd and 34kd (details of procedure not provided).

A plasmid profile of B. thuringiensis var. tenebrionis was provided, however, no designations of plasmid sizes were given.

It was stated that "no cytolytic effects..." of B. thuringiensis var. tenebrionis (designated as SAN 418) "...were found on monkey vero cells with cultures that were grown on the standard commercial production medium. However, it was found that when the cultures

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were grown overnight in a beef heart infusion glucose medium... BTT (SAN 418) showed a relatively low cytolytic symptom on monkey vero cells." [Details of the assay were not provided].

The B. thuringiensis var. tenebrionis strain (designated SA10) was passed five times through nutrient agar slants. At each transfer, the culture was subcultured in liquid nutrient broth, and then subcultured again from the broth into a growth medium that simulated the actual fermentation ingredients medium. Such transfers did not appear to have any significant effect on crystal concentration (determined on a mg/ml basis), on flagellar antigen (i.e., on the 8a8b serotype), or on beta-exotoxin production (negative at all determination times).

Five mice each were injected intraperitoneally with a 1:1000 diluted suspension of SA10 from each of the five transfer preparations. The number of spores injected into each animal was reported as $>10^6$, but actual numbers were not reported. The number of spores/g in the simulated fermentation medium was reported as approx. 10^{10}, and if 0.5 ml of a 1:1000 dilution of this preparation was used, then approximately $5x10^6$ spores were injected into each animal. This should be confirmed or clarified. None of the injected mice died as a result of dosing by the intraperitoneal route, and all mice gained weight during the 7 days following dosing.

Discussion:

1. Sandoz CPC made reference to the original isolation and description of B. thuringiensis var. tenebrionis by A. Krieg et al., (1983, Z. ang. Ent., vol. 96, pp. 500-508), however, it was not clear whether the strain proposed for registration is the same strain or is a different strain, i.e., one that was isolated independently. Sandoz CPC should provide a discussion on the source of the strain they propose to register, and should clarify if their strain SA10 is that which has been described by Krieg, et al.

2. The following information (in addition to the historical information requested above) should be provided by Sandoz CPC to allow for better characterization of their strain:

A. Antibiotic resistance pattern, including erythromycin resistance.

B. Molecular weights of plasmids.

C. Number of spores injected intraperitoneally into mice, and reasons why a 1:1000 diluted preparation was used (The Toxicology Branch suggests that usually $\geq 10^8$ bacteria/animal should be injected).

3. Details of the monkey cell cytotoxicity assay were not provided, and are not needed at this time, since no cytotoxicity was observed after growth in the commercial preparation medium.

4. Data on the presence/absence of non-B.t. microbial contaminants were not provided, and should be done on newly produced batches.